APPLICATIONS

Chiral Separation of Naproxen by High Performance Liquid Chromatography on Lux® i-Amylose-1

James Hseu, Marc Jacob and Simon Lomas
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

In this technical note, we report the chiral separation of racemic Naproxen using the immobilized polysaccharide-based chiral stationary phase Lux i-Amylose-1. Under optimal conditions, the racemic Naproxen was efficiently separated with a resolution factor of 2.33 and a separation factor (alpha) of 1.16. This method can be used for the enantiomeric separation and purity assessment of S-Naproxen, the Active Pharmaceutical Ingredient (API).

Introduction

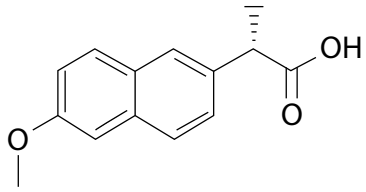
Naproxen is a member of the arylacetic acid group of nonsteroidal anti-inflammatory drugs. Enantiomeric purity of Naproxen is important, as only the S-enantiomer is safe (**Figure 1**). The R-enantiomer is reported to be a liver toxin. Researchers have also shown that the (S) chiral form of the drug Naproxen has 28 times the anti-inflammatory activity of the opposite chiral relative.[1] Based on those findings, it is critical to make sure that the API produced as well as the final drug tablet are enantiomerically pure and only contain the active S-enantiomer.

In this technote, we report the chiral separation of racemic Naproxen using the new Lux i-Amylose-1 immobilized chiral stationary phase.

Materials and Methods

Analyses were performed using an Agilent® 1100 HPLC (Agilent Technologies Santa Clara, CA United States) consisting of a LC quaternary pump system interfaced with a diode array detector (DAD). The Lux i-Amylose-1 column used for analysis was obtained from Phenomenex (Torrance, CA, USA). All solvents were purchased from Honeywell (Morris Plains, NJ, USA) and MilliporeSigma (St. Louis, MO, USA). Racemic Naproxen Catalog No. Y0000399 and S-Naproxen Catalog No. M1275 reference standards were purchased from MilliporeSigma.

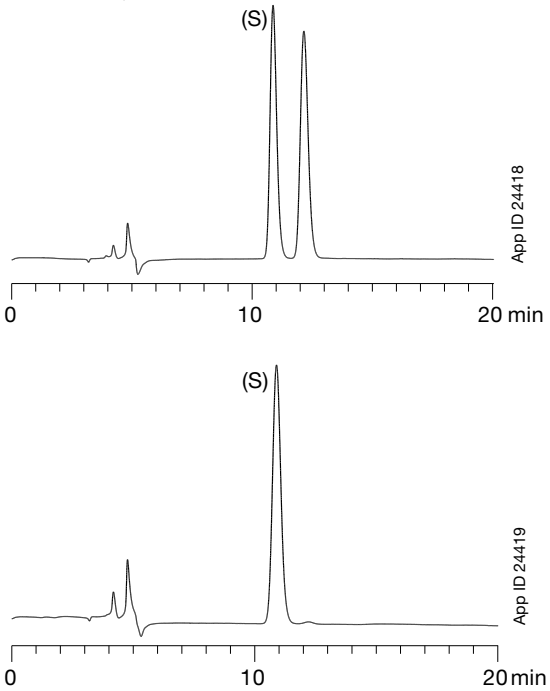
Figure 1. S-Naproxen Chemical Structure



Results and Discussion

Naproxen API has an absolute configuration S as depicted in **Figure 1**. In order to evaluate the enantiomeric purity of the drug substance, pharmaceutical ingredient manufacturers have to be able to separate and quantify enantiomeric content for the unwanted R-enantiomers in the final API. In this technote, we report a method for the enantiomeric separation of Naproxen enantiomers using the Lux i-Amylose-1. As described in the HPLC conditions, the racemic Naproxen was dissolved in the mobile phase at concentration of 1 mg/mL. The prepared sample was injected on an Agilent HPLC system and separation was performed using a mixture of Hexane, Dichloromethane (DCM) and Isopropanol (IPA) with Trifluoroacetic acid (TFA) as additives as described in the HPLC conditions. A representative chromatogram is shown in **Figure 2**. By using pure reference standard S-Naproxen, we confirmed that the S-Naproxen elute first followed by the unwanted R-Enantiomer. The retention times for the S-Naproxen and its R-Enantiomer were 10.87 and 12.15 minutes, respectively. The calculated resolution was 2.33 and the selectivity was 1.16. It is important to notice that the peak asymmetry is quite good with no tailing which should allow easy method validation for API release.

Figure 2. Chiral Separation of Racemic Naproxen and S-Naproxen on Lux 5 µm i-Amylose-1



S-Naproxen Rt	R-Naproxen Rt	Selectivity	Resolution
10.87 min	12.15 min	1.16	2.33

HPLC Conditions:

Column: Lux 5 µm i-Amylose-1
Dimensions: 250 x 4.6 mm
Part No.: 00G-4762-E0
Mobile Phase: n-Hexane/DCM/IPA/TFA (35:65:1:0.1)
Flow Rate: 1 mL/min
Injection: 1 µL
Temperature: Ambient
Detection: UV @ 254 nm
Sample: Racemic Naproxen 1 mg/mL in 1:4 Ethanol/Hexane

Conclusions

The results shown in this technote demonstrate that the immobilized polysaccharide-based chiral stationary phase Lux i-Amylose-1, can be successfully used to perform the enantiomeric purity analysis of S-Naproxen. The resolution for the separation of racemic Naproxen was 2.33, the selectivity was 1.16 and the peak shape obtained were very good for optimal integration and further method validation.

References

1) A. Jenkins and W. A. Hedgepeth, Analysis of Chiral Pharmaceuticals Using HPLC With CD Detection, *Chirality* 17 (2005) 24–29

APPLICATIONS

Lux® Ordering Information

3 µm Minibore, MidBore™, and Analytical Columns (mm)									SecurityGuard™ Cartridges (mm)	
Phases	50 x 2.0	150 x 2.0	100 x 3.0	150 x 3.0	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 2.0*	4 x 3.0*
i-Amylose-1	00B-4761-B0	00F-4761-B0	00D-4761-Y0	00F-4761-Y0	00B-4761-E0	00D-4761-E0	00F-4761-E0	00G-4761-E0	AJ0-8640	AJ0-8641
								for ID:	2.0–3.0 mm	3.2–8.0 mm

5 µm Minibore and Analytical Columns (mm)					SecurityGuard™ Cartridges (mm)
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*
i-Amylose-1	00B-4762-E0	00D-4762-E0	00F-4762-E0	00G-4762-E0	AJ0-8641
				for ID:	3.2–8.0 mm

5 µm Semi-Prep Columns (mm)		SecurityGuard™ Cartridges (mm)
Phases	250 x 10.0	10 x 10.0‡
i-Amylose-1	00G-4762-N0	AJ0-8642
	for ID:	9–16 mm

5 µm Axia™ Packed Preparative Columns (mm)					SecurityGuard™ Cartridges (mm)	
Phases	150 x 21.2	250 x 21.2	250 x 30	250 x 50	15 x 21.2**	15 x 30.0♦
i-Amylose-1	00F-4762-P0-AX	00G-4762-P0-AX	00G-4762-U0-AX	00G-4762-V0-AX	AJ0-8643	AJ0-8644
				for ID:	18–29 mm	30–49 mm

Australia
t: +61 (0)2-9428-6444
f: +61 (0)2-9428-6445
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
f: +43 (0)1-319-1300
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
f: +1 (310) 328-7768
info@phenomenex.com

China
t: +86 400-606-8099
f: +86 (0)22 2532-1033
phen@agela.com

Denmark
t: +45 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
f: +33 (0)1 30 09 21 11
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
f: +49 (0)6021-58830-11
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
f: +91 (0)40-3012 2411
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
f: +39 051 6327555
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
f: +64 (0)9-4780952
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico
t: +1 (800) 541-HPLC
f: +1 (310) 328-7768
info@phenomenex.com

Spain
t: +34 91-413-8613
f: +34 91-413-2290
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
f: +44 (0)1625-501796
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

All other countries
Corporate Office USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

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* SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282
‡ SemiPrep SecurityGuard™ Cartridges require holder, Part No.: AJ0-9281

**HPLC PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8223
SFC PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8617
♦ HPLC PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8277
SFC PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8618

guarantee

If Lux analytical columns (≤ 4.6 mm ID) do not provide at least an equivalent or better chiral separation as compared to a competing column of the same particle size, similar phase and dimensions, return the column with comparative data within 45 days for a FULL REFUND.

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